[ORIGINAL RESEARCH]

In Vitro Percutaneous Absorption of Benzoyl Peroxide from Three Fixed Combination Acne Formulations

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ABSTRACT

Fixed combination therapy in acne is standard of care, and benzoyl peroxide is a common component of a number of fixed-dose combination products available today. Given that benzoyl peroxide can cause concentration-dependent irritation, newer combinations have been developed utilizing lower concentrations (2.5%) in their formulation. These formulations have been shown to provide better tolerability than products with higher benzoyl peroxide concentrations, while offering comparable efficacy. *In vitro* skin permeation studies can be used to determine the relative availability of benzoyl peroxide from different dosage forms. In this *in vitro* percutaneous absorption study, the authors compared three fixed combinations, two with 2.5% benzoyl peroxide and one with 5% benzoyl peroxide. Both 2.5% benzoyl peroxide products (1.2% clindamycin phosphate and 2.5% benzoyl peroxide, and 0.1% adapalene and 2.5% benzoyl peroxide) had similar benzoyl peroxide delivery profiles in terms of efficiency of deposition and total benzoyl peroxide tissue permeation. Although 1.2% clindamycin phosphate and 2.5% benzoyl peroxide delivered the same amount of benzoyl peroxide into the receptor fluid as 1.2% clindamycin phosphate and 5% benzoyl peroxide, it was statistically more efficient in terms of percent applied dose (P=0.002). This suggests a more advanced formulation, as it contains only half the concentration of benzoyl peroxide. All three products showed similar delivery characteristics in terms of the amount of benzoyl peroxide depositing into the dermis. (*J Clin Aesthet Dermatol.* 2013;6(8):19–22.)

Combination therapy is considered standard of care in the management of acne. It is important to select medications that are effective, but also well-tolerated as patients have high expectations but poor adherence.[1] Given that two of the most commonly used acne medications in combination (benzoyl peroxide [BPO] and retinoids) are potentially irritating, this can be a challenge. The most common side effects we see with the topical products are local cutaneous events, such as erythema, dryness, and burning/stinging.[2] With retinoids, irritation is most common in the first two weeks, as the skin learns to tolerate the drug.[3] This initial irritation can often be managed by initiating therapy with a lower concentration retinoid and titrating up.[3,4] Managing irritation associated with BPO is not so straightforward, as its irritation potential relates to the concentration, independent of any adjustment period.[5] Recently, several fixed combination drugs with lower concentrations of BPO (i.e., 2.5% vs. 5%) have been introduced to address this issue. Studies have shown a marked reduction (33%) in mean irritancy scores with one fixed combination product by reducing the concentration of BPO from 5% to 2.5%.[6]

While efficacy is evaluated in clinical trials, drug bioavailability and potency are determined in formulation development. An *in vitro* skin permeation assay is a means of determining relative drug availability, as it accurately reflects the rate determining aspects of drug delivery in most instances.[7]

In this study, *in vitro* skin permeation methodology was utilized to assess the effects of formulation development

DISCLOSURE: Dr. Zeichner has served as an advisory board member, consultant, or investigator for Beiersdorf, Galderma, Medicis, Onset, Ortho Dermatologics, PharmaDerm, Procter and Gamble, Promius Pharma, and Valeant Pharmaceuticals. Drs. Bhatt and Pillai are employees and stockholders of Valeant Pharmaceuticals North America LLC.

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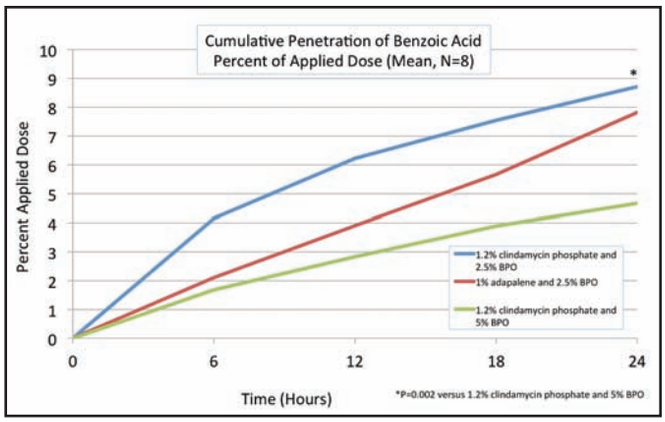

Figure 1. Cumulative receptor phase levels of benzoyl peroxide (expressed as benzoic acid) in percent of applied dose

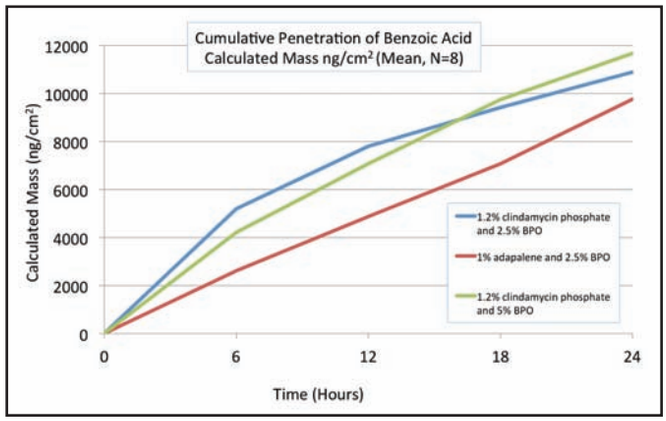

Figure 2. Cumulative receptor phase levels of benzoyl peroxide (expressed as benzoic acid) in total amount delivered per unit area

on the delivery of BPO from three commonly prescribed fixed combination products, two of which contain low concentrations (2.5%) of BPO and one with a higher concentration (5%) of BPO. Products were applied to excised human skin from elective surgery.

METHODS

This *in vitro* percutaneous absorption study was carried out using methodology adapted from the United States Food and Drug Administration (FDA) and the American Association of Pharmaceutical Scientists (AAPS) report.[7] Fixed-dose combination test products (1.2% clindamycin phosphate and 2.5% BPO [Acanya®, Medicis, a division of Valeant Pharmaceuticals], 0.1% adapalene and 2.5% BPO [Epiduo®, Galderma Laboratories], and 1.2% clindamycin phosphate and 5% BPO [Duac®, Steifel Laboratories, Inc.] were all provided by Valeant Pharmaceuticals North America LLC.

Clinically relevant doses (5mg/cm^2) of each test product were applied to dermatomed human skin from a single donor, obtained following elective surgery and prepared within 24 hours of excision. Skin thickness, measured with a snap gauge micrometer ranged from 0.610 to 0.991mm (mean, SD 0.798±0.119, and coefficient of variation of 15%). The dermatomed portion of the skin contains the stratum corneum, epidermis. and small portion of the dermis. The epidermal and dermal layers were separated mechanically for measurement.

Percutaneous absorption was evaluated using skin mounted in Bronaugh flow-through diffusion cells, with a nominal diffusion area of 0.64cm^2. Cells were maintained at a constant temperature of 32°C by the use of recirculating water baths. Fresh receptor solution (phosphate buffered saline [PBS], pH 7.4, containing 0.1% sodium azide and 4% bovine serum albumin) was continuously pumped under the tissue at a nominal flow rate of 0.5mL/hr and collected in six-hour intervals. Receptor phase samples were collected in pre-weighed scintillation vials, with post weights taken at the end of the study.

Following 24-hour exposure, the formulation residing on the stratum corneum was removed by tape-stripping with CuDerm D-Squame (CuDerm Corporation) stripping discs. Topically applied BPO is rapidly metabolized to benzoic acid by the skin.[8–10] The levels of benzoic acid were used in lieu of BPO levels to evaluate penetration into skin. The epidermis, dermis, and receptor phase samples were labeled and frozen prior to analysis of benzoic acid content using liquid chromatography-tandem mass spectrometry (LC/MS/MS). Limits of quantification were 0.098µg/mL (receptor phase) and 0.020µg/mL (tissue samples), due to different extraction methodologies.

Tissue permeation and deposition results were statistically evaluated using unpaired student *t*-test, where significant differences between formulations were defined by a p-value of <0.05, at a 95% confidence interval.

RESULTS: DELIVERY EFFICIENCY

Tissue permeation (receptor phase levels). Tissue permeation over time for the three test products is shown in Figure 1. Tissue permeation after 24 hours ranged from 4.66 to 8.71 percent of applied dose of BPO. Fixed combinations containing 1.2% clindamycin phosphate and 2.5% BPO, and 0.1% adapalene and 2.5% BPO had the highest delivery efficiency (8.71 and 7.81% of applied dose of BPO, respectively, Table 1). The difference between 1.2% clindamycin phosphate and 2.5% BPO, and 1.2% clindamycin phosphate and 5% BPO was significant (P=0.002). Although the amounts of BPO in the three formulations differed, the total amounts of BPO delivered over 24 hours were similar (Figure 2).

Dermal deposition. Dermal deposition of BPO ranged from 0.29 to 0.53 percent of applied dose. The fixed combinations containing 1.2% clindamycin phosphate and 2.5% BPO and 0.1% adapalene and 2.5% BPO had the highest efficiency of dermal BPO deposition (0.42 and 0.53% of applied dose of BPO, respectively, Table 1). There was no statistically significant difference between the three groups.

TABLE 1. Cumulative receptor phase and tissue levels of benzoic acid following 24 hours topical exposure

FORMULATION	RECEPTOR CONTENT AT 24 HOURS MEAN (SD) % COEFFICIENT OF VARIATION (CV)		EPIDERMIS MEAN (SD) % CV		DERMIS MEAN (SD) % CV	
	% APPLIED DOSE	CALCULATED µg/cm² ACTIVE PHARMACEUTICAL INGREDIENT (API) FOR FORMULATION DOSE OF 5mg/cm²	% APPLIED DOSE	CALCULATED µg/cm² API FOR FORMULATION DOSE OF 5mg/cm²	% APPLIED DOSE	CALCULATED µg/cm² API FOR FORMULATION DOSE OF 5mg/cm²
1.2% clindamycin phosphate and 2.5% BPO	8.71 (2.97) 34%	10.9 (3.7) 34%	1.27 (0.73) 58%	1.59 (0.92) 58%	0.42 (0.25) 58%	0.53 (0.31) 58%
0.1% adapalene and 2.5% BPO	7.81 (3.25) 42%	9.76 (4.06) 42%	2.10 (0.77) 37%	2.63 (0.96) 37%	0.53 (0.19) 36%	0.66 (0.24) 36%
1.2% clindamycin phosphate and 5% BPO	4.66 (1.33) 28%	11.7 (3.3) 28%	1.52 (0.36) 23%	3.79 (0.89) 23%	0.29 (0.11) 38%	0.72 (0.28) 38%

Epidermal deposition. Epidermal deposition of BPO ranged from 1.27 to 2.10 percent of applied dose. The fixed combinations containing 1.2% clindamycin phosphate and 5% BPO and 0.1% adapalene and 2.5% BPO had the highest efficiency of epidermal BPO deposition (1.52 and 2.10% of applied dose of BPO, respectively, Table 1). The difference between 1.2% clindamycin phosphate and 2.5% BPO, and 0.1% adapalene and 2.5% BPO was significant (P=0.044).

RESULTS: TOTAL AMOUNT DELIVERED

The total amount of BPO delivered from the test products is dependent on the concentration of BPO in the product as well as the efficiency of delivery. All the amounts are reflected as percentage of applied dose unless otherwise stated.

Tissue permeation (receptor phase levels). The calculated mass of BPO permeating the tissue was similar for all three test products. Numerically, the fixed combinations containing 1.2% clindamycin phosphate and 2.5% BPO and 1.2% clindamycin phosphate and 5% BPO delivered the most BPO (10.9 and 11.7µg/cm², respectively). There was no statistically significant difference between the three groups (Table 1).

Dermal deposition. The calculated BPO dermal deposition ranged from 0.53 to 0.72µg/cm². The fixed combinations containing 1% clindamycin phosphate and 5% BPO and 0.1% adapalene and 2.5% BPO had the highest BPO dermal deposition (0.72 and 0.66µg/cm², respectively, Table 1). However, there was no statistical difference between the three test products.

Epidermal deposition. The calculated BPO epidermal deposition ranged from 1.59 to 3.79µg/cm². The fixed combinations containing 1% clindamycin phosphate and 5% BPO and 0.1% adapalene and 2.5% BPO had the highest BPO epidermal deposition (3.79 and 2.63µg/cm², respectively, Table 1). The difference between 1.2% clindamycin phosphate and 2.5% BPO, and 0.1% adapalene and 2.5% BPO and 1% clindamycin phosphate and 5% BPO was significant (P=0.044 and P<0.001, respectively).

DISCUSSION

The objective of this study was to characterize the *in vitro* percutaneous absorption of BPO from three commonly used topical fixed combination acne treatments following application to excised human skin from elective surgery. Generally, permeation of BPO into the receptor compartment was high. Low dermal and moderate epidermal deposition values were associated with all three study drugs. Since BPO breaks down quite rapidly in skin, the breakdown product, benzoic acid, was used as a marker for BPO.

Two of the study drugs combine clindamycin with BPO, the only difference being in the concentration of BPO (2.5% and 5%) in the formulations. The development of a lower BPO concentration (2.5%) fixed combination product was predicated on a belief that by reducing the concentration of BPO the potential concentration-dependent irritant effects of BPO would be reduced. Indeed, it has been previously shown that there was a 33-percent reduction in potential irritation by halving the concentration of BPO.

It was postulated that the unique formulation of the 1.2% clindamycin phosphate and 2.5% BPO fixed combination would enable levels of BPO available in the skin to be comparable to products containing 5% BPO, thereby potentially allowing for equivalent efficacy. Generally, delivery into the receptor compartments can

be correlated with the amount of unbound drug available. In this study, the 1.2% clindamycin phosphate and 2.5% BPO fixed combination delivered the same amount of BPO into the receptor fluid as the 1.2% clindamycin phosphate and 5% BPO fixed combination over 24 hours. However, the 1.2% clindamycin phosphate and 2.5% BPO fixed combination was twice as efficient in delivering BPO as the product with 5% BPO, despite having only half the concentration of BPO in the formulation. The 1.2% clindamycin phosphate and 2.5% BPO demonstrated a statistically significant, twofold higher percentage of applied dose. In addition, faster delivery of BPO was seen with the 1.2% clindamycin phosphate and 2.5% BPO fixed combination in comparison to either of the comparators, and particularly evident 6 to 12 hours post dosing.

In treating acne, high drug tissue levels are desirable as the site of action is typically within the epidermal and dermal layers. In this study, there were no differences in the total amount of BPO delivered to the dermis by all three products.

No direct, head-to-head clinical comparisons exist to evaluate efficacy among these three products. The two fixed combinations with 2.5% BPO (1.2% clindamycin phosphate and 2.5% BPO and 0.1% adapalene and 2.5% BPO) were shown to have similar BPO delivery profiles in terms of efficiency of deposition and the total amount permeating through the tissue. While direct comparisons are difficult to make, in two large pivotal studies, 1.2% clindamycin phosphate and 2.5% BPO, and 0.1% adapalene and 2.5% BPO appeared comparable in reducing both inflammatory and noninflammatory lesions in patients' moderate acne.[11,12] One split-face study compared the irritation potential of the two products over a 14-day treatment period and showed that irritation potential was more pronounced with 0.1% adapalene and 2.5% BPO, with more patients preferring to continue treatment with 1.2% clindamycin phosphate and 2.5% BPO.[13]

A meta-analysis of 16 randomized controlled trials comparing the efficacy of various fixed-dose clindamycin/BPO combinations was recently published. 1.2% clindamycin phosphate and 2.5% BPO was found to be comparable to clindamycin and 5% BPO in reducing inflammatory lesions and may be superior in treating noninflammatory lesions.[14] The results reported in this *in vitro* percutaneous absorption study is supportive of these findings.

CONCLUSION

This *in vitro* skin permeation study compared the relative availability of BPO from three fixed-dose combination products for acne. All three products had similar BPO delivery profiles, regardless of the percentage of BPO in the formulation. The data suggest that product formulation is just as important as concentration of ingredients, as product with half the concentration of BPO delivered equal amounts of BPO to the dermis.

ACKNOWLEDGMENT

The authors thank Brian Bulley, MSc (Inergy Limited, UK), for assistance with the preparation of the manuscript. Medicis, a division of Valeant Pharmaceuticals, funded Inergy's activities pertaining to this manuscript.

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